

**Amendments to the Claims**

Please cancel Claims 32-34, 57-59, 137 and 138. Please amend Claims 28 and 56.

Please add new Claims 141-148. The Claim Listing below will replace all prior versions of the claims in the application:

**Claim Listing**

1. (Withdrawn) A method of altering the activity of a Sir2 protein comprising the step of altering the NAD-dependent acetylation status of at least one amino acid residue in an acetylated protein.
2. (Withdrawn) The method of Claim 1, wherein the acetylated protein is a nuclear protein.
3. (Withdrawn) The method of Claim 2, wherein the nuclear protein is a histone protein.
4. (Withdrawn) The method of Claim 3, wherein the histone protein is selected from the group consisting of an H2A, H2B, H3 and H4 histone protein.
5. (Withdrawn) The method of Claim 1 wherein the acetylated protein is a cytoplasmic protein.
6. (Withdrawn) The method of Claim 1, wherein the amino acid residue is a lysine amino acid residue.
7. (Withdrawn) The method of Claim 3, wherein the lysine amino acid residue is lysine 9 or lysine 14 of an H3 histone protein.
8. (Withdrawn) The method of Claim 3, wherein the lysine amino acid residue is lysine 16 of an H4 histone protein.
9. (Withdrawn) The method of Claim 1, wherein the alteration in NAD-dependent acetylation status is removal of an acetyl group.
10. (Withdrawn) The method of Claim 1, wherein the Sir2 protein is a Sir2 $\alpha$  protein.

11. (Withdrawn) The method according to Claim 10, wherein the Sir2 $\alpha$  protein has the amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 4, 9, 12, 19 and 26.
12. (Withdrawn) The method according to Claim 10, wherein the Sir2 $\alpha$  protein is encoded by the nucleic acid sequence of SEQ ID NO: 25.
13. (Withdrawn) The method of Claim 10, wherein the Sir2 $\alpha$  protein is a mutant Sir2 $\alpha$  protein selected from the group consisting of G253A, G255A, S257A, I262A, F265A, R266A, G270A, P285A, T336A, H355A, Thr-261, Iso-271, Arg-275 and Asn-345.
14. (Withdrawn) A method of identifying an agent which alters the activity of a Sir2 protein comprising the step of assessing the ability of the agent to alter the NAD-dependent acetylation status of at least one amino acid in an acetylated protein.
15. (Withdrawn) The method of Claim 14, wherein the acetylated protein is a nuclear protein.
16. (Withdrawn) The method of Claim 15, wherein the nuclear protein is a histone protein.
17. (Withdrawn) The method of Claim 14, wherein the acetylated protein is a cytoplasmic protein.
18. (Withdrawn) The method of Claim 14, wherein the ability of the agent to alter the NAD-dependent acetylation status of at least one amino acid in an acetylated protein is assessed by a method, comprising the steps of:
  - (a) combining an acetylated protein with a Sir2 protein, an NAD compound and the agent to be tested, thereby producing a combination;
  - (b) detecting the NAD-dependent acetylation status of an amino acid in the acetylated protein in the combination; and
  - (c) comparing the NAD-dependent acetylation status of an amino acid in the acetylated protein in the combination with the NAD-dependent acetylation status of the amino acid in the acetylated protein in the absence of the agent to be tested,

wherein a difference in the NAD-dependent acetylation status of the amino acid of the acetylated protein in the presence of the agent as compared with the absence of the agent indicates that the agent alters the NAD-dependent acetylation status of at least one amino acid of the acetylated protein.

19. (Withdrawn) The method of Claim 18, wherein the acetylated protein is a nuclear protein.
20. (Withdrawn) The method of Claim 19, wherein the nuclear protein is a histone protein.
21. (Withdrawn) The method of Claim 18, wherein the acetylated protein is a cytoplasmic protein.
22. (Withdrawn) The method of Claim 18, wherein the agent is an agonist of the activity of the Sir2 protein.
23. (Withdrawn) The method of Claim 18, wherein the agent is an antagonist of the activity of the Sir2 protein.
24. (Withdrawn) The method of Claim 18, wherein the alteration of NAD-dependent acetylation status is removal of an acetyl group.
25. (Withdrawn) The method of Claim 20, wherein the histone protein is selected from the group consisting of an H2B, H3 and H4 histone protein.
26. (Withdrawn) The method of Claim 18, wherein the Sir2 protein is a Sir2 $\alpha$  protein.
27. (Withdrawn) The method of Claim 18, wherein the detecting step is performed by electron-spray mass spectroscopy.
28. (Currently Amended) A method of identifying an agent that alters NAD-dependent deacetylation activity of a Sir2 protein~~life span of an organism or a cell~~, comprising the step of measuring NAD-dependent deacetylation activity of a Sir2 protein or a fragment thereof of the Sir2 protein with respect to at least one ~~amino acid~~lysine residue in an acetylated protein ~~in the organism or the cell~~ in the presence of the agent, wherein the

fragment of the Sir2 protein comprises a core domain of Sir2 and includes an amino acid sequence selected from the group consisting of the amino acid sequence represented in at least one of Figs. 2C, 6A, 14A and 19; and SEQ ID NOs. 27 and 28; wherein a decrease in NAD-dependent deacetylation activity of the Sir2 protein with respect to the at least one lysine residue amino acid in the acetylated protein in the organism or the cell in the presence of the agent compared to the NAD-dependent deacetylation activity of the Sir2 protein with respect to the amino acid at least one lysine residue in the acetylated protein in the absence of the agent indicates the agent decreases the life span of the organism or the cell and NAD-dependent deacetylation of at least one lysine residue in the acetylated protein wherein an increase in NAD-dependent deacetylation activity of the Sir2 protein with respect to the amino acid at least one lysine residue in the acetylated protein in the organism or the cell in the presence of the agent compared to the NAD-dependent deacetylation activity of the Sir2 protein with respect to the amino acid in the acetylated protein in the absence of the agent indicates the agent increases the life span of the organism or the cell NAD-dependent deacetylation of at least one lysine residue in the acetylated protein.

29. (Original) The method of Claim 28, wherein the acetylated protein is a nuclear protein.
30. (Original) The method of Claim 29, wherein the nuclear protein is a histone protein.
31. (Original) The method of Claim 28, wherein the acetylated protein is a cytoplasmic protein.
- 32.-41. (Canceled)
42. (Withdrawn) A method of altering the NAD-dependent acetylation status of at least one amino acid residue in an acetylated protein comprising the step of combining the acetylated protein with a Sir2 protein and an NAD compound.
43. (Withdrawn) The method of Claim 42, wherein the acetylated protein is a nuclear protein.
44. (Withdrawn) The method of Claim 43, wherein the nuclear protein is a histone protein.

45. (Withdrawn) The method of Claim 44, wherein the histone protein is selected from the group consisting of an H2B, H3 and H4 histone protein.
46. (Withdrawn) The method of Claim 42, wherein the protein is a cytoplasmic protein.
47. (Withdrawn) The method of Claim 42, wherein the Sir2 protein is a Sir2 $\alpha$  protein.
48. (Withdrawn) A method of identifying an agent which alters mono-ADP-ribosylation of a nuclear protein in an organism, comprising the steps of:
  - a) combining an organism and an agent to be tested;
  - b) determining a level of mono-ADP-ribosylation of a nuclear protein in the organism; and
  - c) comparing the level determined in step (b) with a level of mono-ADP-ribosylation of the nuclear protein in the absence of the agent to be tested,wherein a difference in the level of mono-ADP-ribosylation of the nuclear protein between the presence of the agent and the absence of the agent indicates that the agent alters mono-ADP-ribosylation of the nuclear protein.
49. (Withdrawn) The method of Claim 48, wherein the organism is yeast.
50. (Withdrawn) The method of Claim 48, wherein the organism is *C. elegans*.
51. (Withdrawn) The method of Claim 48, wherein the organism is mammalian.
52. (Withdrawn) The method according to Claim 48, wherein the nuclear protein is a histone protein.
53. (Withdrawn) The method according to Claim 48, wherein the mono-ADP-ribosylation is performed by a Sir2 protein.
54. (Withdrawn) The method according to Claim 53, wherein the mono-ADP-ribosylation is performed by a Sir2 $\alpha$  protein.

55. (Withdrawn) A method of identifying an agent which alters life span of an organism, comprising the steps of:
- combining an organism and an agent to be tested;
  - determining a level of mono-ADP-ribosylation of a nuclear protein in said organism; and
  - comparing the level determined in step (b) with a level of mono-ADP-ribosylation of the nuclear protein in the absence of the agent to be tested,
- wherein a difference in the level of mono-ADP-ribosylation of the nuclear protein between the presence of the agent and the absence of the agent indicates that the agent alters the life span of the organism.
56. (Currently Amended) A method of identifying an agent that alters NAD-dependent deacetylation activity of a Sir2 protein~~life span of an organism or a cell~~, comprising the steps of:
- combining ~~[[the]] an acetylated protein, organism or the cell~~ and the agent to be tested and the Sir2 protein or a fragment of the Sir2 protein, wherein the fragment of the Sir2 protein comprises a core domain of Sir2 and includes an amino acid sequence selected from the group consisting of the amino acid sequence represented in at least one of Figs. 2C, 6A, 14A and 19; and SEQ ID NOs: 27 and 28;
  - measuring NAD-dependent deacetylation activity of ~~[[a]] the Sir2 protein or [[a]]the fragment of the Sir2 protein thereof in the organism or the cell~~ with respect to at least one ~~amino acid~~lysine residue in ~~[[an]]the acetylated protein in said organism; and~~
  - comparing the NAD-dependent deacetylation activity of the Sir2 protein or the fragment of the Sir2 protein with respect to ~~the amino acid~~at least one lysine residue in the acetylated protein in the presence of the agent with the NAD-dependent deacetylation activity of the Sir2 protein or the fragment of the Sir2 protein with respect to ~~the amino acid~~at least one lysine residue in the acetylated protein in the absence of the agent,

wherein a difference in the NAD-dependent deacetylation activity of the Sir2 protein or the fragment of the Sir2 protein with respect to at least one lysine residue in the acetylated protein in the presence of the agent identifies the agent that alters ~~the life span of the organism or the cell~~ NAD-dependent deacetylation of the Sir2 protein.

57.-59. (Canceled)

60. (Previously Presented) The method of Claim 56, wherein the acetylated protein is a nuclear protein.

61. (Original) The method according to Claim 60, wherein the nuclear protein is a histone protein.

62. (Previously Presented) The method of Claim 56, wherein the acetylated protein is a cytoplasmic protein.

63. (Canceled)

64. (Previously Presented) The method according to Claim 56, wherein the Sir2 protein is a Sir2 $\alpha$  protein.

65. (Withdrawn) A method of identifying an agent which alters aging of an organism, comprising the steps of:

- a) combining an organism and an agent to be tested;
- b) determining a level of mono-ADP-ribosylation of a nuclear protein in the organism; and
- c) comparing the level determined in step (b) with a level of mono-ADP-ribosylation of the nuclear protein in the absence of the agent to be tested,

wherein a difference in the level of mono-ADP-ribosylation of the nuclear protein between the presence of the agent and the absence of the agent indicates that the agent alters aging of the organism.

66. (Withdrawn) A method of identifying an agent which alters aging of an organism, comprising the steps of:

- a) combining an organism and an agent to be tested;
- b) determining an NAD-dependent acetylation status of a protein in said organism;  
and
- c) comparing the NAD-dependent acetylation status of the protein in step (b) with an NAD-dependent acetylation status of the protein in the absence of the agent to be tested,

wherein a difference in the NAD-dependent acetylation status of the protein in the presence of the agent and the absence of the agent indicates that the agent alters aging of the organism.

- 67. (Withdrawn) The method of Claim 66, wherein the protein is a nuclear protein.
- 68. (Withdrawn) The method according to Claim 67, wherein the nuclear protein is a histone protein.
- 69. (Withdrawn) The method of Claim 66, wherein the protein is a cytoplasmic protein.
- 70. (Withdrawn) The method according to Claim 66, wherein the NAD-dependent acetylation status of the protein is altered by a Sir2 protein.
- 71. (Withdrawn) The method of Claim 66, wherein the organism is yeast.
- 72. (Withdrawn) The method of Claim 66, wherein the organism is *C. elegans*.
- 73. (Withdrawn) The method of Claim 66, wherein the organism is mammalian.
- 74. (Withdrawn) A method of increasing the life span of an organism, comprising the steps of:
  - a) combining a first organism and an agent to be tested;
  - b) determining a level of mono-ADP-ribosylation of a nuclear protein in the organism;
  - c) comparing the level determined in step (b) with a level of mono-ADP-ribosylation of the nuclear protein in the absence of the agent to be tested, wherein in the



presence of the agent there is an increase in the level of mono-ADP-ribosylation of the nuclear protein; and

- d) administering the agent to a second organism, whereby the lifespan of the second organism increased by the agent.

75. (Withdrawn) A method of increasing the life span of an organism, comprising the steps of:

- a) combining a first organism and an agent to be tested;
- b) determining an NAD-dependent acetylation status of a protein in the organism;
- c) comparing the NAD-dependent acetylation status of the protein in step (b) with an NAD-dependent acetylation status of the nuclear protein in the absence of the agent to be tested, wherein in the presence of the agent there is an increases the deacetylation of the protein; and
- d) administering the agent to a second organism, whereby the lifespan of the second organism increased by the agent.

76.-83. (Canceled)

84. (Withdrawn) A method of decreasing aging of an organism, comprising the steps of:

- a) combining a first organism and an agent to be tested;
- b) determining an NAD-dependent acetylation status of a protein in the organism;
- c) comparing the NAD-dependent acetylation status determined in step (b) with an NAD-dependent acetylation status the protein in the absence of the agent to be tested, wherein in the presence of the agent there is an increase in the deacetylation status of the protein; and
- d) administering the agent to a second organism, wherein the life span of the second organism is decreased by the agent.

85.-92. (Canceled)

93. (Withdrawn) A method of increasing the life span of an organism comprising administering to the organism a mono-ADP-ribosyltransferase or an agonist of a

mono-ADP-ribosyltransferase in an amount effective to increase the life span of the organism.

94. (Withdrawn) A method of increasing the life span of an organism comprising administering to the organism an NAD-dependent deacetylase or an agonist of an NAD-dependent deacetylase.
- 95.-101. (Canceled)
102. (Withdrawn) A method of decreasing aging of an organism comprising administering to the organism an NAD-dependent deacetylase or an agonist of an NAD-dependent deacetylase.
103. (Withdrawn) The method of Claim 102, wherein the NAD-dependent deacetylase is a Sir2 protein.
104. (Canceled)
105. (Withdrawn) A method of inhibiting formation, replication and/or accumulation of rDNA circles in an organism comprising administering to the organism an NAD-dependent deacetylase.
106. (Withdrawn) The method of Claim 105, wherein the organism is yeast.
107. (Withdrawn) The method of Claim 105, wherein the organism is *C. elegans*.
108. (Withdrawn) The method of Claim 105, wherein the organism is mammalian.
109. (Withdrawn) The method of Claim 105, wherein the NAD-dependent deacetylase is a Sir2 protein.
110. (Canceled)
111. (Withdrawn) A method for decreasing recombination between rDNA in an organism comprising administering to the organism an NAD-dependent deacetylase.

112.-115. (Canceled)

116. (Withdrawn) A method of identifying an agent which is an agonist of Sir2 activity, comprising the steps of:
- a) combining a yeast cell and an agent to be tested to form a combination; and
  - b) assessing the presence of red colonies in the combination, wherein the presence of red colonies indicates the agent is an agonist of Sir2 activity.
117. (Withdrawn) A method of identifying an agent which is an antagonist of Sir2 activity, comprising the steps of:
- a) combining a yeast cell and an agent to be tested to form a combination; and
  - b) assessing the presence of white colonies in the combination, wherein the presence of white colonies indicates the agent is an antagonist of Sir2 activity.
118. (Withdrawn) A method of evaluating deacetylation of a substrate in the presence of a Sir2 core domain, and NAD, the method comprising:
- a) providing a mixture comprising a substrate that comprises an acetylated lysine amino acid side chain, a recombinantly produced or isolated protein that comprises SIR2 core domain, and NAD; and
  - b) determining if the substrate is deacetylated.
119. (Withdrawn) The method of claim 118 wherein the protein comprises a human SIR2 core domain.
120. (Withdrawn) The method of claim 118 wherein the mixture comprises  $\text{MgCl}_2$ .
121. (Withdrawn) The method of claim 118 wherein the mixture comprises dithiothreitol (DTT).
122. (Withdrawn) The method of claim 118 wherein the protein is an isolated protein.
123. (Withdrawn) The method of claim 118 wherein the protein is a recombinantly produced protein.

124. (Withdrawn) The method of claim 123 wherein the protein is a fusion protein.
125. (Withdrawn) The method of claim 123 wherein the protein is recombinantly produced in *E. coli*.
126. (Withdrawn) A method of evaluating deacetylation of a substrate in the presence of a Sir2 core domain, and NAD, the method comprising:
- a) providing a mixture comprising a substrate that comprises an acetylated lysine amino acid side chain, a recombinantly produced or isolated protein that comprises a SIR2 core domain comprising SEQ ID NO:4, and NAD; and
  - b) determining if the substrate is deacetylated.
127. (Withdrawn) A method of evaluating deacetylation of a substrate in the presence of a Sir2 core domain, and NAD, the method comprising:
- a) providing a mixture comprising a substrate that comprises an acetylated lysine amino acid side chain, a recombinantly produced or isolated protein that comprises a fragment of a SIR2 protein, and NAD, wherein the fragment of the SIR2 protein comprises SEQ ID NO:38 and SEQ ID NO:28 and NAD-dependent deacetylase activity; and
  - b) determining if the substrate is deacetylated.
128. (Withdrawn) The method of claim 127 wherein the fragment is a fragment of a human SIR protein.
129. (Withdrawn) The method of claim 127 wherein the mixture further comprises an agent to be tested.
130. (Withdrawn) The method of claim 127 wherein the mixture further comprises an agent to be tested.
131. (Previously Presented) The method of Claim 28, wherein the Sir2 protein is an isolated Sir2 protein.

132. (Previously Presented) The method of Claim 131, wherein the isolated Sir2 protein includes a recombinant Sir2 protein.
133. (Previously Presented) The method of Claim 56, wherein the Sir2 protein is an isolated Sir2 protein.
134. (Previously Presented) The method of Claim 133, wherein the isolated Sir2 protein includes a recombinant Sir2 protein.
135. (Previously Presented) The method of Claim 28, wherein the Sir2 protein includes a human Sir2 protein.
136. (Previously Presented) The method of Claim 56, wherein the Sir2 protein includes a human Sir2 protein.
- 137.-138. (Canceled)
139. (Previously Presented) The method of Claim 28, wherein the Sir2 protein is a nuclear protein.
140. (Previously Presented) The method of Claim 56, wherein the Sir2 protein is a nuclear protein.
141. (New) The method of Claim 28, wherein a decrease in NAD-dependent deacetylation activity of the Sir2 protein with respect to at least one lysine residue in the acetylated protein in the presence of the agent compared to the NAD-dependent deacetylation activity of the Sir2 protein with respect to at least one lysine residue in the acetylated protein in the absence of the agent indicates the agent decreases NAD-dependent deacetylation activity of the Sir2 protein.
142. (New) The method of Claim 141, wherein the agent is an antagonist of NAD-dependent deacetylation activity of the Sir2 protein.

143. (New) The method of Claim 28, wherein an increase in NAD-dependent deacetylation activity of the Sir2 protein with respect to at least one lysine residue in the acetylated protein in the presence of the agent compared to the NAD-dependent deacetylation activity of the Sir2 protein with respect to at least one lysine residue in the acetylated protein in the absence of the agent indicates the agent increases NAD-dependent deacetylation activity of the Sir2 protein.
144. (New) The method of Claim 143, wherein the agent is an agonist of NAD-dependent deacetylation activity of the Sir2 protein.
145. (New) The method of Claim 56, wherein the difference in NAD-dependent deacetylation activity of the Sir2 protein or the fragment of the Sir2 protein is an increase in NAD-dependent deacetylation activity and identifies the agent that increases NAD-dependent deacetylation activity of the Sir2 protein.
146. (New) The method of Claim 145, wherein the agent is an agonist of NAD-dependent deacetylation activity of the Sir2 protein.
147. (New) The method of Claim 56, wherein the difference in NAD-dependent deacetylation activity of the Sir2 protein or the fragment of the Sir2 protein is a decrease in NAD-dependent deacetylation activity and identifies the agent that decreases NAD-dependent deacetylation activity.
148. (New) The method of Claim 146, wherein the agent is an antagonist of NAD-dependent deacetylation activity of the Sir2 protein.